

Research Article

Colostrum and blood oxidative stress indices: Effects on growth performance and health in neonatal Holstein calves

Solmaz Namdari,  Mohammad Dadpasand*,  Shahryar Kargar,  Mohammad Reza Jafarzadeh-Shirazi*,  Amir Akhlaghi 

Department of Animal Science, School of Agriculture, Shiraz University, Shiraz, I. R. Iran

ARTICLE INFO

Keywords:

Antioxidant
Reactive oxygen and
nitrogen species

ABSTRACT- This research evaluated the blood and colostrum oxidative stress index (OSi) in newborn Holstein calves and its potential correlations with nutrient intake, growth performance, skeletal development, diarrhea, and pneumonia. Eighty-three neonatal Holstein calves were categorized based on their blood and consumed colostrum OSi levels. There were four treatment groups, i.e., 25 calves consumed low OSi colostrum that had low OSi in the blood taken 24-h after first meal consumption of colostrum (LL), 17 calves consumed low OSi colostrum that had high OSi in the blood (LH), 22 calves consumed high OSi colostrum that had low OSi in the blood (HL), and 19 calves consumed high OSi colostrum that had high OSi in the blood (HH). Upon categorization, the performance and health outcomes of the calves were thoroughly assessed. The results revealed no significant disparities among the treatment groups regarding nutrient intake and skeletal growth. Initial and final body weights, average daily gain (ADG), and feed efficiency had no observable differences. However, substantial differences were evident in the incidence of diarrhea (HH vs. LH, HL vs. LL, and LH vs. LL), pneumonia occurrence (HH vs. LH), and the number of days with body temperatures higher than 39.4 °C were similar across all groups. Moreover, a significant variation was noted in the duration of diarrhea, with the LL group experiencing more days under medication than the other treatment groups. Pronounced variations in calf health, particularly regarding diarrhea and pneumonia, suggesting a potential association between oxidative stress and specific health outcomes in newborn Holstein calves.

INTRODUCTION

Despite considerable advancements in newborn calf management over recent decades, global challenges related to morbidity and mortality persist (Windeyer et al., 2014). Elevated levels of reactive oxygen and nitrogen species (RONS) collectively referred to as oxidative stress (Abuelo et al., 2013), remain a significant concern. In serums, RONS serve as indicators of pro-oxidant production, and their balance is expressed through the pro-oxidant to total antioxidant capacity ratio (RONS/TAC), commonly denoted as oxidative stress index (OSi) (Abuelo et al., 2013). An escalation in this ratio signifies an increased risk of oxidative stress, indicating either heightened pro-oxidant activity or antioxidant depletion (Abuelo et al., 2016). The impact of oxidative stress at birth extends to various facets of calf biology, influencing growth, development, and cellular viability (Abuelo et al., 2019). Oxidative stress contributes to pathological conditions affecting production, reproduction, and overall animal well-being (Lykkesfeldt and Svendsen, 2007). As newborn calves enter extra-uterine life, they become particularly susceptible to diseases due to oxidative stress (Mutinati

et al., 2014). Increased oxidative stress in the blood is a predisposing factor that exacerbates metabolic stresses and immune dysfunction, associated with an increased risk of metabolic and infectious diseases (Abuelo et al., 2019). Recent evaluations demonstrated a significant correlation between diarrhea and a reduction in total antioxidant capacity, an increase in total oxidant status (TOS), and an elevated OSi in neonatal calves (Akyüz and Gökce, 2021).

Increased levels of oxidative stress can occur in newborn calves in the initial weeks after calving (Abuelo et al., 2014), thus leading to a concurrent reduction in their immune response (Cuervo et al., 2021). In the immediate post-birth period, reactive oxygen species (ROS) concentration in calf blood was reportedly 30% higher than in their mothers (Gaál et al., 2006), underscoring the susceptibility of neonatal calves to oxidative challenges. Colostrum is the first essential meal for calves immediately after birth. It contains antioxidants that play a crucial role in mitigating oxidative stress at this critical juncture (Albera and Kankofer, 2011). Furthermore, colostrum intake provides calves with essential immunoglobulins and other beneficial substances, contributing to their overall health (McGuirk and Collins, 2004). Colostrum is a

* Corresponding author: Associate Professor, Department of Animal Science, School of Agriculture, Shiraz University, Shiraz, I.R. Iran
E-mail address: dadpasand@shirazu.ac.ir and jafarzd@shirazu.ac.ir
<https://doi.org/10.22099/iar.2024.50026.1590>

source of antioxidants (Przybylska and Kankofer, 2007). It contains ROS and substantial macromolecules such as lipids and proteins. Notably, colostrum houses a significant population of immune cells, including macrophages that employ the ROS-generating system for bacterial elimination. Two hours after colostrum ingestion, calves exhibit the lowest OSi during the first month of life (Abuelo et al., 2014). Although colostrum has an equal oxidant content compared to regular milk, it has fewer antioxidants (Kankofer and Lipko-Przybylska, 2008). This discrepancy places calves at a higher risk of oxidative stress when consuming colostrum than when taking standard milk (Abuelo et al., 2014).

The presence of antioxidants in colostrum is beneficial in promoting calf health by mitigating the adverse effects of oxidative stress at a cellular level during the initial weeks after calving. Interestingly, the concentration of ROS in the blood declines notably during the initial 3-7 days of a calf's life. However, a subsequent rise in ROS levels occurs between 2 and 3 weeks of age. This temporal pattern is highlighted by lower ROS concentrations on days three to seven compared to day one, followed by an increase after the 2-3 weeks mark (Gaál et al., 2006). This dynamic shift underscores the vulnerability of neonatal calves to oxidative challenges during the early stages of life.

Furthermore, calves born from cows experiencing a higher negative energy balance or oxidative stress tend to exhibit lower body weights at birth (Ling et al., 2018). This observation suggests a potential correlation between the maternal physiological state, characterized by a negative energy balance or oxidative stress, and the neonatal calf's birth weight. It emphasizes the intricate interplay between maternal health and calf outcomes during the perinatal period, further emphasizing the importance of managing oxidative stress for optimal calf health.

Calves exhibit variations in reactive oxygen and nitrogen species and OSi levels in the blood and the colostrum for intake. These distinctions can shape the future health and performance of calves. The available literature lacks explorative research on the health and performance outcomes of calves with differing blood OSi levels, specifically concerning their reception of varying amounts of colostrum with distinct RONS compositions. Therefore, the current research aimed to evaluate OSi levels in the blood and the colostrum through comprehensive measurements and analyses.

MATERIALS AND METHODS

This research was conducted in a local dairy farm. All procedures were approved by the Iranian Council of Animal Care (1995).

Calves, treatments, and management

In this study, Holstein calves were in robust health, and exhibited no signs of disease. Eighty-three calves were systematically housed in individual boxes with straw-based bedding for 60 days. The boxes underwent daily cleaning, and manure removal was conducted regularly. Following birth, the calves were provided with two liters

of warm colostrum via nipple bottles, and this process was repeated every six hours. On the second day after birth, the calves received four liters of transition milk divided into two equal meals—morning and evening, at 08:00 and 18:00, respectively. From day seven onward, the calves received starter feed (approximately containing 21% crude protein and 3.0 Mcal/kg of DM metabolizable energy) in addition to milk allowance. Until day 53, a four-liter milk regimen was maintained, and all calves were eventually weaned on day 60. Small quantities of colostrum from each cow were collected, preserved in tubes, and frozen at -80 °C. Twenty-four hours following the initial colostrum feeding, jugular blood samples were procured into Vacutainer tubes (BD Vacutainer, Franklin Lakes, NJ) containing spray-coated silica. These samples were utilized for determining serum total protein levels using a handheld clinical refractometer (model ATA-2771; Atago Co. Ltd., Tokyo, Japan) according to a method described by Kargar et al. (2020).

Nutrient intake and growth performance

Body weight was assessed on the inaugural day of the study and then every 10 days before the morning feeding, utilizing a calibrated electronic scale (model EES-500; Ettehad Inc., Isfahan, Iran). Average daily gain (ADG) in grams per day (g/d) was calculated based on the difference between body weight measurements at 10-day intervals divided by 10. Daily records of starter consumption and rejection were meticulously logged for each calf to ascertain dry matter intake (DMI), crude protein (CP), ether extract (EE), and metabolizable energy (ME). Feed efficiency (FE) was the ratio of ADG to DMI, encompassing liquid feed DMI and starter feed DMI, according to Kargar et al. (2020). Structural growth indices were comprehensively examined, comprising body height, i.e., distance from the base of the rear feet to shoulder bones, hip width, i.e., the distance between hip bones, and hip height, according to Lesmeister and Heinrichs (2005). In addition, we measured heart girth (chest circumference), withers height (distance from the base of the front feet to withers), and body length (distance between the points of shoulder and rump) on the initial day, and then every ten days until the experiment ended (Pazoki et al., 2017).

Blood and colostrum OSi calculation

To calculate the OSi of blood and colostrum, TAC and TOS were determined with commercial kits. The OSi was then calculated from the formula $Osi = (TOS/TAC) \times 100$ in colostrum and blood samples. Calves were categorized into four groups according to their blood and OSi: LL represented calves with low OSi (below average) in the colostrum and the blood (25 calves). LH indicated calves with low OSi in the colostrum for intake but high OSi (above average) in the blood (17 calves). HL represented calves with high OSi in the colostrum for intake but low OSi in the blood (22 calves). HH indicated calves with high OSi in both the colostrum and the blood (19 calves).

Skeletal growth and health status

The calves underwent daily health assessments, involving evaluations of milk and starter consumption and overall demeanor by a veterinarian. The assessments followed a standard referenced protocol (Larson et al., 1977) that was modified by Heinrichs et al. (2003). General appearance scores were assigned as 1 for normal and alert, 2 for ears drooped, 3 for head and ears drooped, dull eyes, slightly lethargic, 4 for head and ears drooped, dull eyes, lethargic, and 5 for severely lethargic. Fecal scores were determined each day at 07:00, based on fecal consistency, i.e., 1 for normal, 2 for soft to loose, 3 for loose to watery, 4 for watery, mucous, and slightly bloody, and 5 for watery, mucous, and bloody. Rectal temperature (RT) was recorded daily between 12:00 and 14:00 with a digital thermometer (model CT20; EmsiG GmbH, Hamburg, Germany). The thermometer remained in the rectum for approximately 10 seconds. Days with abnormal general appearance (score ≥ 2), fecal score (score ≥ 3), and RT ($x \geq 39.4$ °C, indicating fever) were categorized. These classifications were described as days with abnormal general appearance, fecal score, and RT, respectively (Kargar et al., 2020). Diagnosis of diarrhea and pneumonia was performed by a veterinarian, and treated following standard farm procedures.

Statistical analysis

Data on total nutrient intake (day 1 to day 61), ADG, feed efficiency, and skeletal growth were analyzed using the MIXED procedure of the SAS software (version 9.4; SAS Institute Inc., Cary, NC), involving observations at different times (1- or 10-day period) and as repeated measurements. Each calf was considered a random factor, treatment (T), period (P; 1- or 10-d period), and T \times P as fixed effects. Initial and final body weight data were analyzed using the same model, excluding the period effect. Initial body weight and initial skeletal measurements were included as covariate in the model for the analysis of the body weight and skeletal size, respectively. Autoregressive covariance structure (type 1) was the best fit for these data, determined by the lowest Bayesian information criterion. The significance level was set at $P \leq 0.05$ and trends were declared at $0.05 < P \leq 0.10$.

Models for the occurrence of elevated rectal temperature ($x \geq 39.4$ °C), diarrhea ($x \geq 3$), pneumonia, and need for medication were evaluated by logistic regression using a binomial distribution in the GLIMMIX procedure in SAS. Comparisons of the likelihood for any event in each group relied on the odds ratio. The number of days with elevated rectal temperature ($x \geq 39.4$ °C), frequency and duration of diarrhea ($x \geq 3$) or pneumonia, and administration of medication were evaluated using a Poisson distribution in the GENMOD procedure via SAS software.

RESULTS AND DISCUSSION

Nutrient intake and growth performance

Nutrient intake (day 1 to day 60), growth performance, ADG, and FE were similar among all groups (Table 1).

All of these traits underwent changes as the age of the calves increased (week effect: $P < 0.001$).

Skeletal growth and health status

Table 2 presents skeletal growth data, indicating no discernible differences among the treatments throughout the experiment. Notably, skeletal growth traits exhibited significant changes with calf age. Logistic models for various health attributes (Table 3) showed elevated rectal temperature (≥ 39.4 °C), general appearance score (≥ 2), diarrhea (score ≥ 3), pneumonia, and the need for medication (days 1-60). Treatments did not influence rectal temperature.

Diarrhea significantly varied between HH vs. LH, HL vs. LL, and LH vs. LL groups. Diarrhea in the HH group was 1.5 times more probable than in the LH group. Conversely, calves in the HL and LH groups exhibited a 60% and 80% lower risk of having diarrhea, respectively, compared to the LL group. In the HH vs. LH group, pneumonia occurrence showed significant differences, with a 40% lower risk in the HH group.

Diarrhea medication in HH calves was 70% more successful than in the LL group. HL and LH calves required 1.5 times longer diarrhea medication than the LL group. Interestingly, different colostrum and blood OSi did not affect pneumonia medication in all calf groups. Diarrhea duration was longer in the LL group than in the HL and LH groups, with HH having a longer diarrhea duration than LH. Furthermore, LH exhibited a significantly shorter diarrhea duration than LL and HH, while HL showed a shorter duration than LL. Diarrhea medication in the LL group persisted longer than in other treatments ($P < 0.05$) (Table 4).

Dry matter intake (DMI) was similar across the treatment groups. However, the LH and HL groups were characterized by higher feed efficiency (FE) and increased average daily gain (ADG) and final body weight. Various mechanisms suggested that OSi may affect postnatal growth, metabolism, and overall health of newborn dairy calves (Mutinati et al., 2014). The progressive enhancement in nutrient intake, growth performance, and skeletal growth aligns with advancing age. Escalating free radicals may correlate with accelerated oxidative metabolism, resulting from heightened feed intake and giving solid feed to the growing calves (Gaál et al., 2006). Notably, the antioxidant defense mechanisms maintained a relatively constant concentration of reactive oxygen metabolites in growing calves. This stability reflects a consistently high biological antioxidant potential observed at birth and weaning (Ranade et al., 2014). This discovery contrasts with previous research that age does not affect ROM concentrations (Abuelo et al., 2014).

As expected, animals with high OSi were weak and susceptible to disease, as evident in the HH group, where diarrhea incidence surpassed that of the LH group. Various stressors, including the birthing process, an underdeveloped immune system, contamination risks, and the transition from milk to solid feeds, can be compounded by environmental factors such as poor hygiene and heat stress (Kertz et al., 2017). The intricate connection between intestinal and systemic oxidative

stress indicators remains elusive. Diarrhea remains a pervasive challenge on farms, owing to its multifactorial nature. Effective prevention and control demand a comprehensive understanding of the disease complexities, encompassing multiple pathogens, co-infection, environmental factors, feeding programs, and management practices during the calving period and before disease outbreaks (Cho et al., 2014).

Numerous factors contribute to diarrhea in newborn calves, with negative environmental conditions, maternal influences, inadequate care, feeding conditions, and the calf inability to receive colostrum, thus ranking among the most significant. Viruses, fungi, and bacteria can instigate diarrhea in calves (Aydoğdu et al., 2018).

Interestingly, these factors may contribute to diarrhea more than ROS formation and oxidative stress. This finding contradicts previous studies regarding a decrease in antioxidant levels in the blood of newborn mammals immediately after birth, as observed in human infants. The reduction in the antioxidant defense system can be attributed to ischemia or reperfusion injury at calving, indicated by increased blood lactate levels post-birth (Stohrer et al., 2001). Ischemia-reperfusion injury directly results in ROS formation, endothelial cell damage, increased vascular permeability, and activation of neutrophils, platelets, cytokines, and the complement system (Ferrari and Andrade, 2015).

Table 1. Nutrient intake and growth performance of Holstein calves

Traits	Treatment (T)				SEM	P-value		
	LL*	LH	HL	HH		T	Period (P)	T × P
Nutrient intake (d 1 to 60)								
Starter feed intake								
Dry matter, g/d	451	490	520	460	61.255	0.840 ^{NS**}	0.001	0.937 ^{NS}
Crude protein, g/d	98	106	112	99	13.231	0.840 ^{NS}	0.001	0.937 ^{NS}
Ether extract, g/d	12	13	14	12	1.592	0.840 ^{NS}	0.001	0.937 ^{NS}
Metabolizable energy, Mcal/d	1.32	1.43	1.52	1.35	0.179	0.840 ^{NS}	0.001	0.937 ^{NS}
Total feed intake								
Dry matter, g/d	985	1023	1054	994	61.255	0.840 ^{NS}	0.001	0.937 ^{NS}
Crude protein, g/d	220	228	234	221	13.231	0.840 ^{NS}	0.001	0.937 ^{NS}
Ether extract, g/d	147	148	149	148	1.592	0.840 ^{NS}	0.001	0.937 ^{NS}
Metabolizable energy, Mcal/d	4.03	4.14	4.23	4.05	0.179	0.840 ^{NS}	0.001	0.937 ^{NS}
Growth performance								
Body weight, kg								
Initial (d 1)	37.8	37.2	37.3	37.7	0.872	0.947 ^{NS}	—	—
Final (d 60)	72.9	76.8	77.6	73.8	1.592	0.089	—	—
Average daily gain (d 1 to 60), g/d	590	654	669	605	23.766	0.065	0.001	0.975 ^{NS}
Feed efficiency (d 1 to 60)	0.61	0.66	0.67	0.63	0.016	0.081	0.001	0.802 ^{NS}

*LL: Calves with low oxidative stress index (OSi) (less than the average) in consumed colostrum and low OSi in blood.

LH: Calves with low OSi in consumed colostrum and high OSi (above the average) in blood.

HL: Calves with high OSi in consumed colostrum and low OSi in blood.

HH: Calves with high OSi in consumed colostrum and high OSi in blood.

SEM: standard error of means.

** NS: not significant.

Table 2. Skeletal growth parameters of Holstein calves (day 1 to day 60)

Traits (cm)	Treat (T)				SEM	P-value		
	LL*	LH	HL	HH		T	Period (P)	T × P
Heart girth	85.0	85.9	85.2	85.0	0.400	0.382 ^{NS**}	0.001	0.168 ^{NS}
Stature height	80.7	80.9	81.2	80.8	0.223	0.309 ^{NS}	0.001	0.446 ^{NS}
Body length	50.6	50.8	50.9	50.8	0.163	0.469 ^{NS}	0.001	0.432 ^{NS}
Body barrel	93.8	93.6	94.1	93.9	0.358	0.810 ^{NS}	0.001	0.921 ^{NS}
Hip height	82.8	82.4	82.8	82.8	0.232	0.516 ^{NS}	0.001	0.609 ^{NS}
Hip width	16.3	16.3	16.5	16.3	0.125	0.363 ^{NS}	0.001	0.347 ^{NS}

*LL: Calves with low oxidative stress index (OSi) (less than the average) in consumed colostrum and low OSi in blood.

LH: Calves with low OSi in consumed colostrum and high OSi (above the average) in blood.

HL: Calves with high OSi in consumed colostrum and low OSi in blood.

HH: Calves with high OSi in consumed colostrum and high OSi in blood.

** NS: not significant.

Table 3. Logistic regression analysis for rectal temperature, diarrhea, and pneumonia in Holstein calves (day 1 to day 60)

Variable and comparison	Estimate	SE	P-value	Odds ratio	95% CI	
					Lower	Upper
Rectal temperature						
HH* vs. HL	-0.044	0.199	0.823 ^{NS**}	0.956	0.647	1.414
HH vs. LH	-0.334	0.199	0.093 ^{NS}	0.716	0.484	1.058
HH vs. LL	-0.194	0.188	0.303 ^{NS}	0.824	0.569	1.192
HL vs. LH	-0.289	0.189	0.122 ^{NS}	0.749	0.516	1.086
HL vs. LL	-0.149	0.178	0.402 ^{NS}	0.861	0.607	1.222
LH vs. LL	0.140	0.178	0.432 ^{NS}	1.150	0.811	1.632
Diarrhea occurrence						
HH vs. HL	0.375	0.337	0.266 ^{NS}	1.455	0.750	2.823
HH vs. LH	0.949	0.441	0.031	2.584	1.088	6.138
HH vs. LL	-0.428	0.276	0.122 ^{NS}	0.652	0.379	1.121
HL vs. LH	0.574	0.455	0.207 ^{NS}	1.776	0.728	4.333
HL vs. LL	-0.803	0.298	0.007	0.448	0.250	0.804
LH vs. LL	-1.377	0.411	0.001	0.252	0.133	0.565
Pneumonia occurrence						
HH vs. HL	-0.088	0.186	0.636 ^{NS}	0.916	0.635	1.321
HH vs. LH	-0.393	0.186	0.035	0.675	0.468	0.973
HH vs. LL	-0.213	0.177	0.229 ^{NS}	0.808	0.570	1.145
HL vs. LH	-0.305	0.175	0.082 ^{NS}	0.737	0.522	1.040
HL vs. LL	-0.125	0.166	0.451 ^{NS}	0.882	0.637	1.222
LH vs. LL	0.179	0.166	0.279 ^{NS}	1.197	0.864	1.657
Medication occurrence						
Diarrhea						
HH vs. HL	-0.149	0.311	0.631 ^{NS}	0.861	0.468	1.586
HH vs. LH	-0.455	0.364	0.211 ^{NS}	0.634	0.310	1.296
HH vs. LL	0.566	0.264	0.032	1.762	1.048	2.959
HL vs. LH	-0.306	0.364	0.401 ^{NS}	0.736	0.361	1.504
HL vs. LL	0.715	0.264	0.006	2.045	1.218	3.434
LH vs. LL	1.021	0.325	0.001	2.777	1.467	5.255
Pneumonia						
HH vs. HL	0.064	0.191	0.737 ^{NS}	1.066	0.733	1.551
HH vs. LH	0.337	0.191	0.079 ^{NS}	1.401	0.962	2.040
HH vs. LL	0.216	0.181	0.232 ^{NS}	1.241	0.870	1.770
HL vs. LH	0.272	0.181	0.133 ^{NS}	1.314	0.920	1.877
HL vs. LL	0.152	0.170	0.372 ^{NS}	1.164	0.834	1.626
LH vs. LL	-0.120	0.171	0.479 ^{NS}	0.886	0.634	1.239

*LL: Calves with low oxidative stress index (OSi) (less than the average) in consumed colostrum and low OSi in blood.

LH: Calves with low OSi in consumed colostrum and high OSi (above the average) in blood.

HL: Calves with high OSi in consumed colostrum and low OSi in blood.

HH: Calves with high OSi in consumed colostrum and high OSi in blood.

SE: Standard error

** NS: not significant.

Table 4. Poisson regression for rectal temperature, diarrhea, and pneumonia in Holstein calves (day 1 to day 60)

Item	Treat				SEM	P-value
	LL	LH	HL	HH		
Days with elevated rectal temperature (≥39.4)	3.0	3.5	2.6	2.5	0.130	0.321
Diarrhea						
Frequency	0.6	0.2	0.3	0.4	0.376	0.189
Duration, d	1.6 ^c	0.4 ^a	0.7 ^{ab}	1.1 ^{bc}	0.252	0.001
Medication, d	1.9 ^b	0.7 ^a	1.0 ^a	1.1 ^a	0.217	0.002
Pneumonia						
Frequency	0.8	0.9	0.7	0.7	0.249	0.816
Duration, d	3.5	4.1	3.1	2.8	0.121	0.174
Medication, d	3.4	3.8	2.9	2.7	0.124	0.290

*LL: Calves with low oxidative stress index (OSi) (less than the average) in consumed colostrum and low OSi in blood.

LH: Calves with low OSi in consumed colostrum and high OSi (above the average) in blood.

HL: Calves with high OSi in consumed colostrum and low OSi in blood.

HH: Calves with high OSi in consumed colostrum and high OSi in blood.

a-c: In each row, there was no statistically significant difference in the averages of treatments with common clauses ($P > 0.05$)

SE: Standard error

In another study, concentrations of L-lactate in the rumen and feces of diarrheic calves were higher than those of healthy calves, but this difference was not observed in serum and urine. D-lactate concentration in the rumen, feces, blood, and urine of calves with diarrhea was higher than in healthy calves (Ewaschuk et al., 2004). Research demonstrated oxidative stress in animal models experiencing diarrhea (Zeeshan et al., 2021). Suppression of oxidative stress is reportedly a potential strategy to alleviate diarrhea in rodents (Song et al., 2011). However, the relationship between oxidative stress and diarrhea is not universally agreed upon, with reports suggesting that oxidative stress is unaffected by non-malnutrition diarrhea (Granot et al., 2001).

Despite the LL calves exhibiting low OSi, the occurrence of diarrhea in this group exceeded than those of the LH and HL groups. The likelihood of needing diarrhea medication was higher in the LH and HL groups. This is attributed to the superior average daily gain (ADG) and feed efficiency (FE) of the HL and LH groups, indicative of enhanced performance, growth, and health status. The prolonged duration of diarrhea medication in the HH group, as opposed to the LL group, result from non-responsiveness to treatment in some HH calves, leading to treatment cessation by the veterinarian, while health parameter recordings continued. The direct association between oxidative stress occurrence and calf diarrhea remains inadequately defined to date. Diarrhea medication was longer in HH than LL, since some calves in the HH group were not responsive to medication. While veterinarians usually stop the treatment, we continued to record health parameters.

In the LH group, pneumonia occurrence was higher than in the HH group, but medication tended to be significantly effective in the HH group, and rectal temperature tended to be significant in HH but lower than LH. Oxidative stress is a predisposing factor for numerous health disorders, including sepsis, mastitis, acidosis, ketosis, enteritis, pneumonia, and respiratory diseases (Lykkesfeldt and Svendsen, 2007). It is noteworthy to mention that apart from the excessive ROS accumulation during an acute inflammatory response, individuals with certain metabolic/endocrine disorders, such as high body weight (BW), also bear a heightened ROS burden (Niemann et al., 2017). Generally, the chronic disease-induced ROS burden may not be potent enough to eliminate pathogens, but its consistent presence could eventually lead to cellular or tissue damage, increasing the likelihood of pathogen invasion in the lungs and resulting in pneumonia (Wang et al., 2019). In an animal study, increased NADPH oxidase (NOX) activity was observed alongside reduced activity of antioxidant enzymes such as SOD, catalase, and glutathione in white fat tissue (Furukawa et al., 2017).

Epidemiological studies revealed that pneumonia is a serious threat, particularly in individuals with high BW, showcasing an increased incidence and severity of the disease (Fisher-Hoch et al., 2013). In our experiment, despite the lower OSi in the LH group, the probability of pneumonia was higher because of the higher feed efficiency (FE) and final BW compared to the HH group. Several studies showed that an imbalance between lipid peroxides and antioxidants in pneumonia might damage pulmonary endothelium (Doelman and Bast, 1990).

Oxidative stress is believed to seriously impact the pathogenesis of various lung diseases, through direct effects and interference in the molecular mechanisms controlling lung inflammation (MacNee, 2000). In respiratory tract infections, neutrophils are taken to pulmonary tissues to eliminate invading microorganisms through phagocytosis (Lykkesfeldt and Svendsen, 2007). Tissue-damaging products secreted by neutrophils, such as reactive nitrogen intermediates and nitric oxides, modulate both acute and chronic inflammatory reactions. Nitric oxide acts as a mediator of capillary dysfunction and macromolecular leakage. The reaction between superoxide radicals and nitric oxide produces the non-radical oxidant peroxynitrite, causing severe damage to pulmonary tissues (Wessely-Szponder et al., 2004). When exposed to appropriate stimuli, phagocytes generate large quantities of superoxide radicals, deleterious precursors of more reactive species that contribute to pulmonary damage. Moreover, the administration of antioxidants reportedly decreased lung lipid peroxidation after endotoxin infusion (Demling et al., 1988). In calves with bronchopneumonia, superoxide radical levels were ten times higher than in healthy calves, while the antioxidant enzyme superoxide dismutase activity was lower (Ledwozyw and Stolarczyk, 1992). Free radicals and TBARS (thiobarbituric acid reactive substances) reportedly increased during inflammatory lung disorders, such as pneumonia (Lang et al., 2002). In children with acute pneumonia, TBARS concentrations increased, thus resulting in oxidative stress (Cemek et al., 2006). Blood malondialdehyde (MDA), serum ceruloplasmin, and total bilirubin levels were higher than in healthy children, while superoxide dismutase (SOD), glutathione peroxidase, β -carotene, retinol, vitamin E, vitamin C, and glutathione levels were lower in children with pneumonia. All antioxidant activities decreased in children with acute pneumonia. Oxidative stress reportedly increased, while enzymatic and non-enzymatic antioxidant activities significantly decreased in children with acute pneumonia (Cemek et al., 2006). Many researchers measured major enzymatic or non-enzymatic antioxidants in patients with pneumonia (Reyes et al., 2002). A decrease in the activity of superoxide dismutase in calves with pneumonia correlated with high circulatory concentrations of superoxide radicals (Ledwozyw and Stolarczyk, 1992). In addition, a significant decrease in glutathione (GSH) concentration was observed in human patients (Pacht et al., 1991). Marked increases in OS markers (TBARS and lactoperoxidase (LPO)) occurred in calves with bronchopneumonia, similar to previous findings. Antioxidant systems (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) enzymes and glutathione (GSH concentrations) decreased in animals with chronic pneumonia. Oxidative stress plays a role in various pathological conditions of livestock, thus affecting both production and animal welfare. Notably, common diseases like pneumonia in pigs (Lauritzen et al., 2003) reportedly changed the redox balance (Lykkesfeldt and Svendsen, 2007).

CONCLUSION

These findings indicated that calves with varying oxidative stress indices in the blood and colostrum for intake did not exhibit substantial differences in terms of growth

performance and food consumption. However, there were differences among these calves in the incidence, medication, and duration of diseases. This divergence can be attributed to factors beyond oxidative stress, including environmental conditions, maternal health status, parity, and the immune system. Future research can assess maternal cow conditions before calving, considering the aforementioned factors, and explore the long-term impact of different OSi on the health of calves from the neonatal period to weaning. The transient nature of the differences in blood and colostrum OSi within the first 24 hours of life raises questions about their duration of persistence. Subsequent tests should aim to determine how many days these differences persist. If permanent, assessing whether it affects calf performance becomes a critical aspect of future research.

FUNDING

This study was funded by Shiraz University.

CRedit AUTHORSHIP CONTRIBUTION STATEMENT

Mohammad Dadpasand: Conceptualization: supervision, methodology, validation, and review & editing. Solmaz Namdari: Investigation, methodology, data curation, formal analyses, and writing original draft. Shahryar Kargar: Conceptualization, methodology, validation, writing original draft, and writing – review & editing. Mohammad Reza Jafarzadeh Shirazi: Methodology, validation, and writing – review & editing. Amir Akhlaghi: Validation and writing – review & editing.

DECLARATION OF COMPETING INTEREST

The authors confirm that there are no conflicts of interest regarding this publication.

ETHICAL STATEMENT

All procedures were approved by the Iranian Council of Animal Care (1995).

DATA AVAILABILITY

The authors declare that the datasets are available from the corresponding author on request.

ACKNOWLEDGMENTS

The authors would like to thank Raz Lab for blood samples analyses and farm staffs for providing all facilities needed for this study. Special thanks also go to Meysam Kanani for all his collaboration and helps.

REFERENCES

- Abuelo, A., Hernández, J., Benedito, J. L., & Castillo, C. (2013). Oxidative stress index (OSi) as a new tool to assess redox status in dairy cattle during the transition period. *Animal*, 7, 1374-1378. <https://doi.org/10.1017/S1751731113000396>.
- Abuelo, Á., Pérez-Santos, M., Hernández, J., & Castillo, C. (2014). Effect of colostrum redox balance on the oxidative status of calves during the first 3 months of life and the relationship with passive immune acquisition. *Veterinary Journal*, 199, 295-299. <https://doi.org/10.1016/j.tvjl.2013.10.032>.
- Abuelo, A., Gandy, J., Neuder, L., Brester, J., & Sordillo, L. (2016). Markers of oxidant status and inflammation relative to the development of claw lesions associated with lameness in early lactation cows. *Journal of Dairy Science*, 99, 5640-5648. <https://doi.org/10.3168/jds.2015-10707>.
- Abuelo, A., Hernández, J., Benedito, J. L., & Castillo, C. (2019). Redox biology in transition periods of dairy cattle: Role in the health of periparturient and neonatal animals. *Antioxidant*, 8, 20. <https://doi.org/10.3390/antiox8010020>
- Akyüz, E., & Gökce, G. (2021). Neopterin, procalcitonin, clinical biochemistry, and hematology in calves with neonatal sepsis. *Tropical Animal Health and Production*, 53, 1-8. <https://doi.org/10.1007/s11250-021-02779-z>.
- Albera, E., & Kankofer, M. (2011). The comparison of antioxidative/oxidative profile in blood, colostrum and milk of early post-partum cows and their newborns. *Reproduction of Domestic Animals*, 46, 763-769. <https://doi.org/10.1111/j.1439-0531.2010.01737.x>.
- Aydoğdu, U., Yildiz, R., Güzelbekteş, H., Naseri, A., Akyüz, E., & Şen, İ. (2018). Effect of combinations of intravenous small volume hypertonic sodium chloride, acetate Ringer, sodium bicarbonate, and lactate Ringer solutions along with oral fluid on the treatment of calf diarrhea. *Polish Journal of Veterinary Sciences*, 21, 273-280.
- Cemek, M., Çaksen, H., Bayiroğlu, F., Cemek, F., & Dede, S. (2006). Oxidative stress and enzymic-non-enzymic antioxidant responses in children with acute pneumonia: Cellular biochemistry and its modulation by active agents or disease. *Cell Biochemistry and Function*, 24, 269-273. <https://doi.org/10.1002/cbf.1220>.
- Cho, Y., & Yoon, K. (2014). An overview of calf diarrhea - infectious etiology, diagnosis, and intervention. *Journal of Veterinary Science*, 15, 1-17. <https://doi.org/10.4142/jvs.2014.15.1.1>.
- Cuervo, W., Sordillo, L. M., & Abuelo, A. (2021). Oxidative stress compromises lymphocyte function in neonatal dairy calves. *Antioxidant*, 10, 255. <https://doi.org/10.3390/antiox10020255>.
- Demling, R., Lalonde, C., & Seekamp, A. (1988). Endotoxin causes hydrogen peroxide-induced lung lipid peroxidation and prostanoid production. *Archives of Surgery*, 123, 1337-1341. <http://doi:10.1001/archsurg.1988.01400350051007>.
- Doelman, C. J., & Bast, A. (1990). Oxygen radicals in lung pathology. *Free Radical Biology and Medicine*, 9, 381-400.
- Ewaschuk, J. B., Naylor, J. M., Palmer, R., Whiting, S. J., & Zello, G. A. (2004). D-lactate production and excretion in diarrheic calves. *Journal of Veterinary Internal Medicine*, 18, 744-747. <https://doi.org/10.1111/j.1939-1676.2004.tb02615.x>.
- Ferrari, R. S., & Andrade, C. F. (2015). Oxidative stress and lung ischemia-reperfusion injury. *Oxidative Medical and Cellular Longevity*, 2015, 1-14. <https://doi.org/10.1155/2015/590987>.

- Fisher-Hoch S. P., Mathews, C. E., & McCormick, J. B. (2013). Obesity, diabetes and pneumonia: The menacing interface of non-communicable and infectious diseases. *Tropical Medicine and International Health*, 18, 1510-1519. <https://doi.org/10.1111/tmi.12206>.
- Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., Nakayama, O., Makishima, M., Matsuda, M., & Shimomura, I. (2017). Increased oxidative stress in obesity and its impact on metabolic syndrome. *Journal of Clinical Investigation*, 114, 1752-1761. [10.1172/JCI121625](https://doi.org/10.1172/JCI121625).
- Gaál, T., Ribiczeyné-Szabó, P., Stadler, K., Jakus, J., Reiczigel, J., Kövér, P., Mezes, M., & Sümeghy, L. (2006). Free radicals, lipid peroxidation and the antioxidant system in the blood of cows and newborn calves around calving. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 143, 391-396. <https://doi.org/10.1016/j.cbpb.2005.12.014>.
- Granot, E., Binsztok, M., Fraser, D., Deckelbaum, R., & Weizman, Z. (2001). Oxidative Stress is not enhanced in non-malnourished infants with persistent diarrhea. *Journal of Tropical Pediatrics*, 47, 284-287. <https://doi.org/10.1093/tropej/47.5.284>
- Heinrichs, A., Jones, C., VanRoekel, L., & Fowler, M. (2003). Calf Track: A system of dairy calf workforce management, training, and evaluation and health evaluation. *Journal of Dairy Science*, 86, 115.
- Iranian Council of Animal Care. (1995). *Guide to the care and use of experimental animals*, Vol. 1. Isfahan, Iran: Isfahan University of Technology.
- Kankofer, M., & Lipko-Przybylska, J. (2008). Physiological antioxidative/oxidative status in bovine colostrum and mature milk. *Acta Veterinaria*, 58, 231-239. <https://doi.org/10.2298/AVB0803231K>.
- Kargar, S., Roshan, M., Ghoreishi, S., Akhlaghi, A., Kanani, M., Shams-Abadi, A. A., & Ghaffari, M. (2020). Extended colostrum feeding for 2 weeks improves growth performance and reduces the susceptibility to diarrhea and pneumonia in neonatal Holstein dairy calves. *Journal of Dairy Science*, 103, 8130-8142. <https://doi.org/10.3168/jds.2020-18355>
- Kertz, A. F., Hill, T. M., Quigley, J. D., Heinrichs, A. J., Linn, J. G., & Drackley, J. K. (2017). A 100-year review: Calf nutrition and management. *Journal of Dairy Science*, 100, 10151-10172. <https://doi.org/10.3168/jds.2017-13062>.
- Lang, J. D., McArdle, P. J., O'Reilly, P. J., & Matalon, S. (2002). Oxidant-antioxidant balance in acute lung injury. *Chest*, 122, 314-320. https://doi.org/10.1378/chest.122.6_suppl.314S.
- Larson, L. L., Owen, F. G., Albright, J. L., Appleman, R. D., Lamb, R. C., & Muller, L. D. (1977). Guidelines toward more uniformity in measuring and reporting calf experimental data. *Journal of Dairy Science*, 60, 989-991.
- Lauritzen, B., Lykkesfeldt, J., Skaanild, M. T., Angen, Q., Nielsen, J. P., & Friis, C. (2003). Putative biomarkers for evaluating antibiotic treatment: An experimental model of porcine *Actinobacillus pleuropneumoniae* infection. *Research in Veterinary Science*, 74, 261-270. [https://doi.org/10.1016/S0034-5288\(03\)00028-6](https://doi.org/10.1016/S0034-5288(03)00028-6)
- Ledwozyw, A., & Stolarczyk, H. (1992). The involvement of polymorphonuclear leukocytes in the pathogenesis of bronchopneumonia in calves. VI. Superoxide dismutase and lipoprotein lipase activities. *Acta Veterinaria Hungarica*, 40, 267-277.
- Lesmeister, K. E., & Heinrichs, A. J. (2005). Effects of adding extra molasses to a texturized calf starter on rumen development, growth characteristics, and blood parameters in neonatal dairy calves. *Journal of Dairy Science*, 88, 411-418. [https://doi.org/10.3168/jds.S0022-0302\(05\)72702-8](https://doi.org/10.3168/jds.S0022-0302(05)72702-8)
- Ling, T., Hernandez-Jover, M., Sordillo, L. M., & Abuelo, A. (2018). Maternal late-gestation metabolic stress is associated with changes in immune and metabolic responses of dairy calves. *Journal of Dairy Science*, 101, 6568-6580. <https://doi.org/10.3168/jds.2017-14038>.
- Lykkesfeldt, J., Svendsen, O. (2007). Oxidants and antioxidants in disease: oxidative stress in farm animals. *Veterinary Journal*, 173, 502-511. <https://doi.org/10.1016/j.tvjl.2006.06.005>.
- MacNee, W. (2000). Oxidants/antioxidants and COPD. *Chest*, 117, 303-317. https://doi.org/10.1378/chest.117.5_suppl.1.303S-a
- McGuirk, S. M., & Collins, M. (2004). Managing the production, storage, and delivery of colostrum. *Veterinary Clinics of North America: Food Animal Practice*, 20, 593-603.
- Mutinati, M., Pantaleo, M., Roncetti, M., Piccinno, M., Rizzo, A., & Sciorsci, R. L. (2014). Oxidative stress in neonatology: A review. *Reproduction of Domestic Animals*, 49, 7-16. <https://doi.org/10.1111/rda.12230>.
- Niemann, B., Rohrbach, S., Miller, M. R., Newby, D. E., Fuster, V., & Kovacic, J. C. (2017). Oxidative stress and cardiovascular risk: Obesity, diabetes, smoking, and pollution: Part 3 of a 3-part series. *Journal of the American College of Cardiology*, 70, 230-251. <https://doi.org/10.1016/j.jacc.2017.05.043>
- Pacht, E. R., Timerman, A. P., Lykens, M. G., & Merola, J. (1991). Deficiency of alveolar fluid glutathione in patients with sepsis and the adult respiratory distress syndrome. *Chest*, 100, 1397-1403.
- Pazoki, A., Ghorbani, G. R., Kargar, S., Sadeghi-Sefidmazgi, A., Drackley, J. K., & Ghaffari, M. H. (2017). Growth performance, nutrient digestibility, ruminal fermentation, and rumen development of calves during transition from liquid to solid feed: Effects of physical form of starter feed and forage provision. *Animal Feed Science and Technology*, 234, 173-185. <https://doi.org/10.1016/j.anifeedsci.2017.06.004>
- Przybylska, J., Albera, E., & Kankofer, M. (2007). Antioxidants in bovine colostrum. *Reproduction in Domestic Animals*, 42, 402-409. <https://doi.org/10.1111/j.1439-0531.2006.00799.x>